

RELATIVE CAPABILITIES OF SARCOPLASMIC RETICULUM IN FAST AND SLOW MAMMALIAN SKELETAL MUSCLES

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SUMMARY

1. The calcium uptake capabilities of the sarcoplasmic reticulum (SR) of the fast-twitch muscles extensor digitorum longus (EDL) and tibialis anterior (TA) of the rat and the extensor digitorum longus of the cat have been compared with the same capabilities of the slow-twitch soleus muscles of the rat and cat.

2. For the rat the V_{\max} values of sarcoplasmic reticulum from tibialis anterior, extensor digitorum longus and from soleus muscles were 50, 51, and 10 $\mu\text{mole Ca}^{2+}/\text{g}$ per minute, respectively.

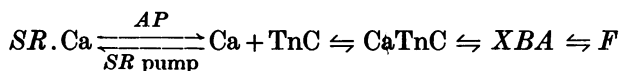
3. For the extensor digitorum longus and soleus muscles of the cat the V_{\max} values were 34 and 5.6 $\mu\text{mole Ca}^{2+}/\text{g}$ per minute, respectively.

4. These data were compared with mechanical data as reported in the literature for the same muscles. The relative calcium uptake capabilities of sarcoplasmic reticulum from slow and fast muscles corresponded closely to the relative rates of relaxation of these muscles.

INTRODUCTION

Although it has long been known that rates of relaxation are markedly different in fast and slow twitch muscles, the mechanisms which account for this difference have not been established. One possibility is that relaxation time reflects the rate of calcium removal from the contractile material and that differences in rates of calcium uptake by the sarcoplasmic reticulum (SR) could account for the difference in relaxation times (Sandow, 1970).

The model of excitation-contraction coupling which this hypothesis assumes and which is compatible with current knowledge can be described symbolically by the following steps



where the action potential (*AP*) releases calcium from the sarcoplasmic reticulum which diffuses through the sarcoplasm and binds to troponin-C (*TnC*) which then permits cross-bridge attachment (*XBA*) and force (*F*) production. The Ca^{2+} will remain bound to troponin until the sarcoplasmic Ca^{2+} level is reduced by uptake into the sarcoplasmic reticulum through the action of the sarcoplasmic reticulum calcium pump. If this is the rate limiting step then relaxation rate should be related to Ca^{2+} uptake rate by the sarcoplasmic reticulum. It is possible, of course, that some other step is the slow step and hence rate limiting for relaxation.

Few attempts have been made to quantitatively examine the relation between sarcoplasmic reticulum calcium uptake and relaxation rate. The report which comes closest to providing the necessary information is that of Fiehn & Peter (1971). In this study the ratio (fast/slow) of the half relaxation times was 5.7 while the ratio (fast/slow) of the rate of calcium uptake (defined as uptake rate times yield) was 7.1. Note that the ratios are inverted because it is assumed that half-relaxation time is inversely related to the rate of calcium loss from the sarcoplasm. Although the relative rates of calcium uptake and relaxation time are in reasonable agreement, and support the assumption that the differences in relaxation times seen in slow and fast twitch muscles are related to differing capabilities of the sarcoplasmic reticulum in the two types of muscle, the study by Fiehn & Peter suffers from: (1) uncertainties about the purity of the isolated sarcoplasmic reticulum fraction and; (2) the use of yield data to estimate the relative amounts of sarcoplasmic reticulum in the muscle being studied. Although their preparations were free of the contaminating enzymes tested for, other contaminating enzymes and proteins could have been present. Also, the use of yield to estimate sarcoplasmic reticulum concentrations is dangerous since only about 10% of the sarcoplasmic reticulum in muscle is obtained from homogenates by centrifugation (Ogawa, 1970; Solaro & Briggs, 1974).

In the report which follows we have chosen to measure the rate of calcium uptake by muscle homogenates. Using this method we can estimate the functional capabilities of sarcoplasmic reticulum without isolating a pure fraction or estimating the amount of sarcoplasmic reticulum in muscle and thus circumvent these problems. In this method Ca^{2+} uptake is limited to the sarcoplasmic reticulum in the homogenate by studying Ca^{2+} uptake at low Ca^{2+} levels (1–10 μM) in the presence of 5 mM azide to eliminate the participation of skeletal muscle mitochondria in Ca^{2+} uptake (Harigaya, Ogawa & Sugita, 1968; Samaha & Gergely, 1965) and in the presence of oxalate to amplify the activity of the sarcoplasmic reticulum. Further details concerning the methods and its validation are presented in the paper by Solaro & Briggs (1974).

METHODS

Adult cats of both sexes and adult male rats were used in these studies. The fast twitch muscles studied were the extensor digitorum longus (EDL) of rats and cats and the tibialis anterior (TA) of rats. The slow twitch muscles studied were the soleus of the rat and cat. The muscles were quickly dissected, placed in ice-cold isotonic saline, blotted dry and weighed. The homogenate was formed by adding 6 volumes of a 300 mM sucrose, 10 mM imidazole (pH 7.0) solution per gram of muscle and subjecting the mixture to 30 sec homogenization with a Sorvall Homogenizer.

The concentration of the muscle homogenate during calcium uptake studies was 1.43 mg wet muscle/ml. bath for fast muscles but doubled to 2.86 mg wet muscle/ml. bath for slow muscle to enhance its calcium uptake to easily measured values. This did not jeopardize the comparison between fast and slow muscles since calcium uptake was found to be linearly dependent upon homogenate concentration over this range. Calcium uptake by the diluted homogenate was measured at 25° C and pH 7.0 in 100 mM-KCl, 20 mM imidazole, 0.2 mM EGTA, 10 mM oxalate, 10 mM Na azide, 5 mM ATP, 5 mM-MgCl₂, 0.05 $\mu\text{C}/\text{ml}$. $^{45}\text{CaCl}_2$ and CaCl₂ at either 0.20, 0.18, 0.14 or 0.10 mM. Ionic calcium concentrations were calculated for this solution as previously described (Solaro & Shiner, 1976) taking into consideration the Ca^{2+} binding to the ATP present.

With the above incubation conditions calcium uptake is limited to vesicles of the fragmented sarcoplasmic reticulum (Solaro & Briggs, 1974). Uptake was stopped by filtration through 0.45 μm Millipore filters. The incubation bath was sampled at 0 and 1.0 min and frequently at 0.5, 1.5 and 2.0 min to monitor the velocity of calcium uptake at different times.

Relative amounts of fragmented sarcoplasmic reticulum in the fast and slow twitch muscles were estimated by the method described by Solaro & Briggs (1974). This method involves determination of steady-state accumulation of calcium oxalate by sarcoplasmic reticulum vesicles in muscle homogenates. Steady-state filling was determined at 37° C in a medium similar to that described above except that EGTA was 1.6 mM, CaCl₂ was 0.64 mM and 6 mM creatine phosphate was added to regenerate ATP. Samples were taken at several times to verify that a steady-state level had been reached.

RESULTS

In table 1 are recorded typical values of velocities of calcium uptake during $\frac{1}{2}$ min intervals of a 2 min experiment. With the tibialis anterior muscle the velocity of calcium uptake declined with the continuous decrease in calcium concentration in the incubation medium. The lower calcium uptake velocities observed in the slow-twitch soleus muscles were generally maintained during the experimental period but fluctuations in measured values were common.

In Fig. 1 the velocities of calcium uptake by homogenates of rat fast and slow twitch muscles are shown for different ionic calcium concentrations. Lineweaver-Burke plots are also shown. The V_{max} values for the tibialis anterior and extensor digitorum longus were 51 and 50 μmole calcium per gram of muscle per minute respectively. The V_{max} for the rat

soleus was $10 \mu\text{mole Ca}^{2+}/\text{g} \times \text{min}$. The ratio of V_{max} (fast/slow) is approximately 5. The K_m values for the tibialis anterior and extensor digitorum longus were 1.51 and 1.46 mM respectively and 0.61 mM for the soleus.

TABLE 1. Calcium concentrations and velocities of calcium uptake during $\frac{1}{2}$ min periods in 2 min experiments involving fast twitch tibialis anterior and slow twitch (soleus) muscles

| | Rates of Ca^{2+} uptake by sarcoplasmic reticulum | | | |
|--------------------|--|----------------------------------|---|--|
| | Sampling time (min) | Total calcium concentration (mM) | Ionic calcium concentration (μM) | Velocity of Ca^{2+} uptake ($\mu\text{mole Ca}^{2+}/\text{g} \cdot \text{min}$) |
| Rat muscle | | | | |
| Tibialis anterior | 0.0 | 0.200 | 7.60 | — |
| (1.43 mg/ml. bath) | 0.5 | 0.173 | 3.80 | 37.3 |
| | 1.0 | 0.150 | 2.30 | 32.2 |
| | 1.5 | 0.134 | 1.65 | 22.9 |
| | 2.0 | 0.122 | 1.35 | 16.1 |
| Soleus | 0.0 | 0.200 | 7.60 | — |
| (2.86 mg/ml. bath) | 0.5 | 0.192 | 6.20 | 5.3 |
| | 1.0 | 0.184 | 5.00 | 6.2 |
| | 1.5 | 0.173 | 3.80 | 7.4 |
| | 2.0 | 0.165 | 3.20 | 5.8 |

The relation between calcium concentration and calcium uptake rate for the cat extensor digitorum longus and soleus muscles is shown in Fig. 2. Also shown are Lineweaver-Burke plots of the data. The V_{max} for the cat extensor digitorum longus is $34 \mu\text{mole Ca}^{2+}$ per gram muscle per minute and for the soleus is $5.5 \mu\text{mole/g} \times \text{min}$. The ratio of V_{max} values (fast/slow) is approximately 6.

Table 2 shows the calcium oxalate capacities of the rat and cat skeletal muscle homogenates which is an indirect method of estimating the relative amounts of sarcoplasmic reticulum present in muscles. The oxalate capacities of each muscle were determined under conditions that assured that the calcium loading of the sarcoplasmic reticulum vesicles had reached a steady state. The capacities of the rat and cat fast twitch muscles were found to be three and two-times greater, respectively, than the corresponding slow twitch soleus muscles.

DISCUSSION

If differences in relaxation times between fast and slow twitch muscle are to be accounted for by differences in the rate of calcium uptake by the sarcoplasmic reticulum, it must be assumed that the intrinsic mechanism

for relaxation is the same in both muscle types (Harigaya *et al.* 1968). Also, since the rates of calcium uptake by the sarcotubular vesicles were measured in the presence of oxalate it is assumed that these data are applicable to the *in vivo* function of the sarcoplasmic reticulum where no oxalate is present. Evidence has been provided that sarcoplasmic reticulum calcium transport in oxalate-free systems and in the presence of oxalate is carried out by the same transport system (Weber, Herz & Reiss, 1964*a, b*) and at the same initial rate (Harigaya *et al.* 1968; Inesi & Scarpa, 1972; Ogawa, 1970).

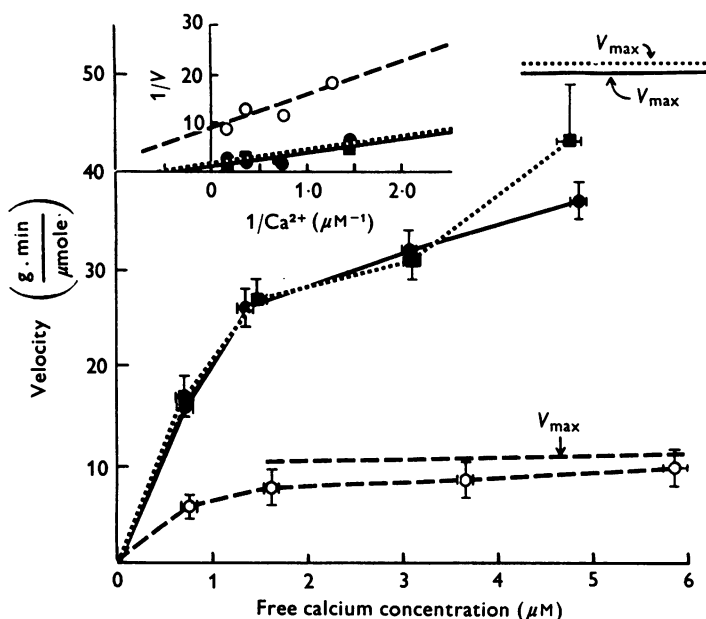


Fig. 1. Velocities of initial calcium uptake plotted against free calcium concentration in rat fast twitch (tibialis anterior, ●—●(8); and extensor digitorum longus, ■.....■(5)) and slow twitch (soleus, ○—○(10)) skeletal muscle homogenates. Each point represents the mean velocity, vertical bars depict the s.e. of mean for velocity values and horizontal bars depict the s.e. of mean for free calcium values. Horizontal lines indicate the V_{\max} values for each muscle. The number in parentheses is the number of muscles studied at each point for that muscle. Insert shows the linear correlation lines determined for the reciprocals of Ca^{2+} concentration and velocity of calcium uptake ($1/V$). V_{\max} and K_m values were determined from these Lineweaver-Burke plots.

Other investigators using isolated fragmented sarcoplasmic reticulum preparations from various muscles of guinea-pigs (Fiehn & Peter, 1971) and rabbits (Sreter & Gergely, 1964; Sreter, 1969) have also reported higher rates of calcium uptake with fast twitch than with slow twitch

muscles. The value of their quantitative comparisons is limited, however, since both the relative purity and concentration of the sarcoplasmic reticulum are unknown.

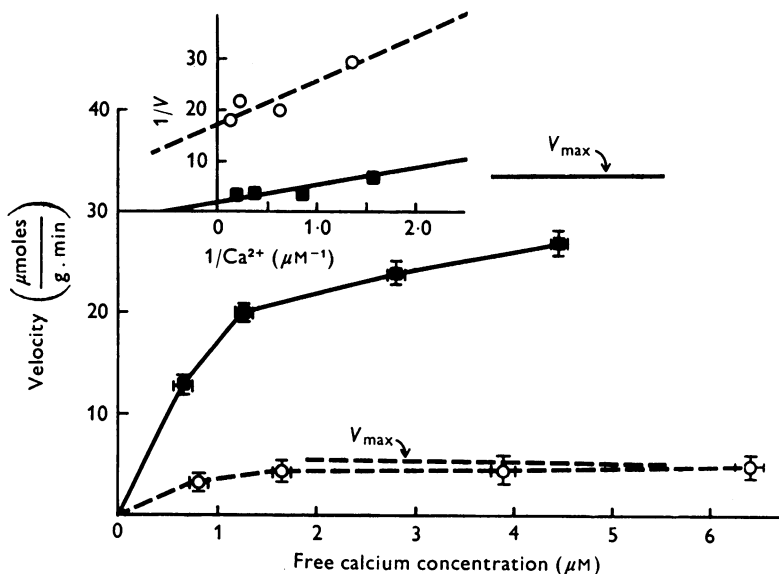


Fig. 2. Velocities of initial calcium uptake plotted against free calcium concentration in cat fast twitch (extensor digitorum longus, ■—■) and slow twitch (soleus, ○—○) skeletal muscle homogenates. Each point represents the mean velocity of three experiments, vertical bars indicate the s.e. of mean for velocity values and horizontal bars depict the s.e. of mean for free calcium values. Horizontal lines indicate the V_{\max} values for each muscle. Insert shows the linear correlation lines determined for the reciprocals of Ca^{2+} concentration and velocity of calcium uptake ($1/V$). V_{\max} and K_m values were determined from these Lineweaver-Burke plots.

TABLE 2. Steady-state calcium uptake by fast twitch and slow twitch skeletal muscle homogenates. Given are means \pm s.e. of mean with the number of experiments in parentheses

| Muscle | Steady-state calcium uptake by muscle homogenates | |
|---------------------------|---|----------------|
| | Calcium uptake | |
| | ($\mu\text{mole Ca}^{2+}/\text{g wet muscle}$) | |
| | Rat | Cat |
| Tibialis anterior | 95 ± 5 (16) | — |
| Extensor digitorum longus | 97 ± 7 (5) | 82 ± 5 (9) |
| Soleus | 33 ± 2 (16) | 42 ± 2 (9) |

The ratio of mechanical relaxation (half-relaxation times) is 6.6 for the rat (soleus/extensor digitorum longus) (Close, 1967) and 4.2 for the cat (soleus/flexor digitorum longus) (Buller, Eccles & Eccles, 1960) and compares favourably with the V_{\max} ratios (fast/slow) reported in this study of 5 and 6 for the rat and cat respectively. Exact agreement would be unlikely since a comparison is being made between calcium uptake in a homogenate system and mechanical activity in an intact system. Although these data fail to exclude the possibility that differences in relaxation rates are due to differences in calcium uptake rates they do not exclude the possibility that relaxation rates are determined by some other step such as dissociation of attached cross-bridges.

The observed difference, between fast and slow twitch muscles, in the rates of calcium uptake by sarcoplasmic reticulum in whole homogenates could be due to a difference in: (1) the amount of sarcoplasmic reticulum per unit weight of muscle; (2) the rate of calcium uptake per unit weight of sarcoplasmic reticulum; or (3) both. The relative amounts of sarcoplasmic reticulum in fast and slow twitch muscles can be only roughly estimated. It was noted in the introduction that Fiehn & Peter (1971) estimated the amount of sarcoplasmic reticulum in fast and slow muscle from the yields of sarcoplasmic reticulum from homogenates of the muscles. On this basis they estimated that the amount of sarcoplasmic reticulum in guinea-pig vastus lateralis muscle is 1.64 times that in soleus muscle. Harigaya *et al.* (1968), also using yield data, estimated the sarcoplasmic reticulum concentration in rabbit soleus muscle to be half that of the rabbit semitendinosus. Using calcium capacities of homogenates (Table 2) we estimate the sarcoplasmic reticulum ratios for fast/slow muscle to be 2.9 for the rat muscles studied and 1.9 for the cat muscles. These rough estimates of sarcoplasmic reticulum concentrations suggest that our observed difference in the rates of calcium uptake by sarcoplasmic reticulum in homogenates cannot be completely accounted for by differences in sarcoplasmic reticulum concentration. As suggested by others (Fiehn & Peter, 1971) the sarcoplasmic reticulum from slow muscle may accumulate calcium at a slower rate than sarcoplasmic reticulum from fast muscle because of differences in the intrinsic rate of the transport mechanism or because of a lower density of the transport site in the sarcoplasmic reticulum membrane.

Fiehn & Peter found the K_m for calcium uptake to be $4 \mu\text{M}$ for both fast and slow guinea-pig muscle. These K_m values are considerably higher than the $1.5 \mu\text{M}$ observed for the sarcoplasmic reticulum from the fast muscles of the cat and rat and the $0.6 \mu\text{M}$ observed for the soleus muscles from the cat and rat. The failure of Fiehn & Peter to consider the effect of ATP on the ionic calcium level, however, more than accounts for the

discrepancy. Using the Mg^{2+} , Ca^{2+} , H^+ and K^+ formation constants to ATP as given by Solaro & Shiner (1976), the ionic calcium concentration would be $0.2 \times 10^{-6} M$ when the total concentration of calcium in the medium is $4 \mu M$, the K_m reported by Fiehn & Peter (1971). Species difference may explain why Fiehn & Peter found no difference in the K_m values for fast and slow muscle.

In conclusion, the possibility that half-relaxation times are primarily determined by the withdrawal of Ca^{2+} from myofilaments by sarcoplasmic reticulum is not excluded by this data. The difference in relaxation times between fast and slow twitch muscles could be accounted for by differences in the amount and properties of the sarcoplasmic reticulum.

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